ChromaFlash™ Chromatin Extraction Kit

Base Catalog # P-2001

PLEASE READ THIS ENTIRE USER GUIDE BEFORE USE

Uses: The ChromaFlash™ Chromatin Extraction Kit is suitable for isolating chromatin or DNA-protein complex from mammalian cells or tissues in a simple and rapid format. Chromatin prepared by this kit can be used in a variety of chromatin immunoprecipitation methods. It is the optimal method for chromatin required by Epigentek's one-hour ChIP method using the ChromaFlash™ One-Step ChIP Kit (P-2025) or ChromaFlash™ One-Step Magnetic ChIP Kit (P-2026). The isolated chromatin can also be used in other chromatin-related applications such as in vitro protein-DNA binding assays and nuclear enzyme assays.

Starting Material and Input amount: Starting materials can include various tissue or cell samples such as cells from flask or microplate cultured cells, fresh and frozen tissues, etc. The amount of cells and tissues for each preparation can be 1 x 10^5 to 5 x 10^6 cells and 10 mg to 200 mg, respectively. For optimal preparation, the input amount should be 1 to 5 x 10^6 cells or 50 to 200 mg tissues. A total of 100 standard extractions (use 1 X10^6 cells or 50 mg of tissue per extraction) can be performed with this kit. Yield of chromatin is approximately 4 µg per 10^6 cells or per 50 mg tissues.

Precautions: To avoid cross-contamination, carefully pipette the sample or solution into the tube/vials. Use aerosol-barrier pipette tips and always change pipette tips between liquid transfers. Wear gloves throughout the entire procedure. In case of contact between gloves and sample, change gloves immediately.

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KIT CONTENTS

Component	100 Preparations P-2001-100	Storage Upon Receipt
10X Lysis Buffer	11 ml	RT
Extraction Buffer	11 ml	RT
Chromatin Buffer	11 ml	RT
Protease Inhibitor Cocktails (1000X)*	110 µl	4°C
User Guide	1	RT

** Spin the solution down to the bottom prior to use.*

SHIPPING & STORAGE

The kit is shipped on frozen ice packs at 4°C.

Upon receipt: (1) Protease Inhibitor cocktails at 4°C; (2) Store remaining components at room temperature.

All components of the kit are stable for 6 months from the date of shipment, when stored properly.

Note: Check if any buffers contain salt precipitates before use. If so, shake the buffer until the salts are re-dissolved.

MATERIALS REQUIRED BUT NOT SUPPLIED

- ☐ Vortex mixer
- ☐ Dounce homogenizer
- ☐ Centrifuge including desktop centrifuge (up to 14,000 rpm)
- ☐ Pipettes and pipette tips
- ☐ 1.5 ml microcentrifuge tubes
- ☐ 15 ml conical tube
- ☐ Cells or tissues
- ☐ Cell culture medium
- ☐ 37% formaldehyde (if cross-linked)
- ☐ 1.25 M Glycine solution (if cross-linked)
- ☐ 1X PBS
- ☐ Distilled water

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GENERAL PRODUCT INFORMATION

Quality Control: Each lot of the ChromaFlash™ Chromatin Extraction Kit is tested against predetermined specifications to ensure consistent product quality. Epigentek guarantees the performance of all products in the manner described in our product instructions.

Product Warranty: If this product does not meet your expectations, simply contact our technical support unit or your regional distributor. We also encourage you to contact us if you have any suggestions about product performance or new applications and techniques.

Safety: Suitable lab coat, disposable gloves, and proper eye protection are required when working with this product.

Product Updates: Epigentek reserves the right to change or modify any product to enhance its performance and design. The information in this User Guide is subject to change at any time without notice. Thus, only use the User Guide that was supplied with the kit when using that kit.

Usage Limitation: The ChromaFlash™ Chromatin Extraction Kit is for research use only and is not intended for diagnostic or therapeutic application.

A BRIEF OVERVIEW

Chromatin immunoprecipitation (ChIP) offers an advantageous tool for studying protein-DNA interaction. With ChIP, the experimenter can determine if a specific protein binds to the specific sequences of a gene in living cells by combining with PCR (ChIP-PCR), microarray (ChIP-chip), or sequencing (ChIP-Seq) techniques. For example, the measurement of the amount of methylated histone H3 at lysine 9 (meH3-K9) associated with a specific gene promoter region under various conditions can be achieved through a ChIP-PCR assay, while recruitment of meH3-K9 to the promoters on a genome-wide scale can be detected by ChIP-chip. In particular, the ChIP method with specific antibodies directly against various transcriptional factors is widely demanded.

For performing ChIP, chromatin or DNA-protein complex in cells or tissues should be first isolated. The ChromaFlash™ Chromatin Extraction Kit addresses the inconvenience and time consuming issues of existing chromatin preparation methods by introducing the following features:

- Extremely fast procedure: the entire procedure from cell/tissue sample to ready-to-use chromatin is less than 60 minutes.
- Convenient and flexible: the kit is suitable for preparing both native chromatin and cross-linked chromatin from monolayer or suspension cells, or from tissues.
- Unsheared chromatin makes it customizable for various analysis workflows that require either intact or fragmented chromatin, including ChIP, in vitro protein-DNA interaction analysis, nuclear enzyme assay, etc.

PRINCIPLE & PROCEDURE

The ChromaFlash™ Chromatin Extraction Kit contains all reagents required for carrying out successful chromatin extraction directly from mammalian cells or tissues. Cell membranes of the sample, with or without cross-linking, are broken down using the provided lysis buffer. Chromatin or DNA-protein complex is then extracted with the extraction buffer. The extracted chromatin can then be diluted with chromatin buffer and stored at the appropriate temperature.

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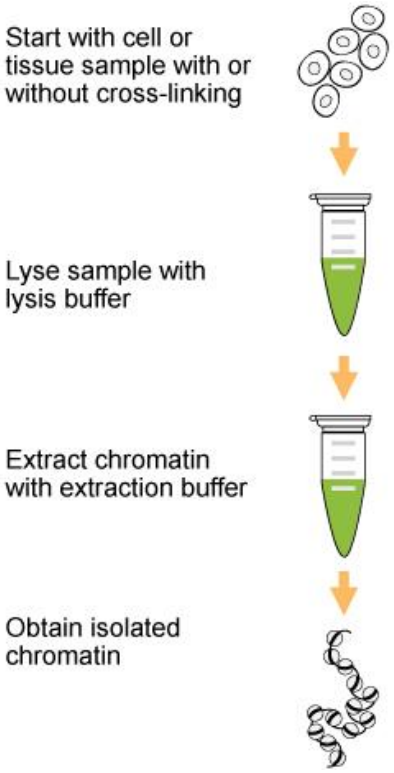
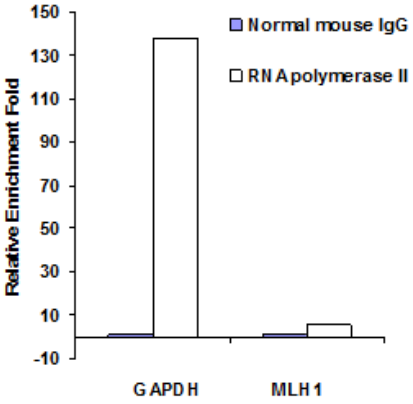

Start with cell or tissue sample with or without cross-linking
Lyse sample with lysis buffer
Extract chromatin with extraction buffer
Obtain isolated chromatin

Schematic procedure of the ChromaFlash™ Chromatin Extraction Kit



ChIP analysis of RNA polymerase II enriched in GAPDH and MLH1 promoters with chromatin extract prepared from formaldehyde fixed colon cancer cells ($2x10^5$) using the ChromaFlash™ Chromatin Extraction Kit

ASSAY PROTOCOL

For the best results, please read the protocol in its entirety prior to starting your experiment.

Starting Materials

Monolayer cells: $1x10^5$ to $5x10^6$ cells per preparation.

Suspension cells: $1x10^5$ to $5x10^6$ cells per preparation.

Tissues: 10 mg to 200 mg per preparation.

1. Preparation of Working Buffers and Solutions

a. Prepare **Working Lysis Buffer** by adding 1 ml of **10X Lysis Buffer** and 6 µl of **Protease Inhibitor Cocktail** to every 9 ml of distilled water.

b. Prepare **Working Extraction Buffer** by adding 1 µl of **Protease Inhibitor Cocktail** to every 1 ml of **Extraction Buffer**

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2. Cell Collection and Cross-Linking

For Monolayer or Adherent Cells:

a. Grow cells (treated or untreated) to 80%-90% confluence on a 100 mm plate, then trypsinize and collect them into a 15 ml conical tube. Count the cells in a hemocytometer.

b. Centrifuge the cells at 1000 rpm for 5 min. Discard the supernatant.

c. Wash cells with 10 ml of PBS once by centrifugation at 1000 rpm for 5 min. Discard the supernatant.

Note: For cells that are not cross-linked, go directly to Step 3d after Step 2c.

d. Add 9 ml fresh cell culture medium containing formaldehyde with a final concentration of 1% (i.e., add 270 µl of 37% formaldehyde to 10 ml of cell culture medium) to cells.

e. Incubate at room temperature (20-25°C) for 10 min on a rocking platform (50-100 rpm).

For Suspension Cells:

a. Collect cells (treated or untreated) into a 15 ml conical tube. Count cells in a hemocytometer.

b. Centrifuge the cells at 1000 rpm for 5 min. Discard the supernatant.

c. Wash cells with 10 ml of PBS once by centrifugation at 1000 rpm for 5 min. Discard the supernatant.

Note: For cells that are not cross-linked, go directly to Step 3d after Step 2c.

d. Add 9 ml fresh cell culture medium containing formaldehyde with a final concentration of 1% (i.e., add 270 µl of 37% formaldehyde to 10 ml of cell culture medium) to cells.

e. Incubate at room temperature (20-25°C) for 10 min on a rocking platform (50-100 rpm).

For Tissues:

a. Put the tissue sample into a 60 or 100 mm plate. Remove unwanted tissue such as fat and necrotic material from the sample.

b. Weigh the sample and cut the sample into small pieces (1-2 mm^3) with a scalpel or scissors.

Note: For tissues that are not cross-linked, go directly to Step 2j after Step 2b.

c. Transfer tissue pieces to a 15 ml conical tube.

d. Prepare cross-link solution by adding formaldehyde to cell culture medium with a final concentration of 1%. (e.g., add 270 µl of 37% formaldehyde to 10 ml of culture medium).

e. Add 1 ml of cross-link solution for every 40 mg tissues.

f. Incubate at room temperature for 15-20 min on a rocking platform.

g. Add 1 ml of 1.25 M glycine for every 9 ml of cross-link solution.

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h. Mix and centrifuge at 800 rpm for 5 min. Discard the supernatant.

i. Wash cells with 10 ml of ice-cold PBS once by centrifugation at 800 rpm for 5 min. Discard the supernatant.

j. Transfer tissue pieces to a Dounce homogenizer.

k. Add 1 ml **Working Lysis Buffer** for every 200 mg tissues.

l. Disaggregate tissue pieces by 10-20 strokes.

m. Transfer homogenized mixture to a 15 ml conical tube and centrifuge at 3000 rpm for 5 min at 4°C. If total mixture volume is less than 2 ml, transfer mixture to a 2 ml vial and centrifuge at 5000 rpm for 5 min at 4°C. Then go directly to Step 3g.

3. Cell Lysis and Chromatin Extraction

a. Add 1 ml of 1.25 M glycine for every 9 ml of cross-link solution.

b. Mix and centrifuge at 1000 rpm for 5 min.

c. Remove medium and wash cells once with 10 ml of ice-cold PBS by centrifuging at 1000 rpm for 5 min.

d. Add **Working Lysis Buffer** to re-suspend the cell pellet (200 µl/1x10^6 cells for adherent cells and 100 µl/1x10^6 cells for suspension cells).

e. Transfer cell suspension to a 1.5 ml vial and incubate on ice for 10 min.

f. Vortex vigorously for 10 sec and centrifuge at 5000 rpm for 5 min.

g. Carefully remove supernatant.

h. Add **Working Extraction Buffer** to re-suspend the chromatin pellet (50 µl/1x10^6 cells, 500 µl maximum for each vial).

i. Incubate the sample on ice for 10 min and vortex occasionally.

j. Resuspend the sample and sonicate 2 X 20 seconds to increase chromain extraction. Allow the sample to cool on ice between sonication pulses for 30 seconds. As an example, sonication can be carried out with a microtip attached to Branson 450 sonifier, setting at 25% power output.

k. Centrifuge at 12,000 rpm at 4°C for 10 min.

l. Transfer supernatant to a new vial.

m. Add **Chromatin Buffer** at a 1:1 ratio (e.g., add 100 µl of **Chromatin Buffer** to 100 µl of supernatant).

The chromatin solution can now be used immediately or stored at –80°C after aliquoting appropriately until further use. Avoid multiple freeze/thaw cycles.

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TROUBLESHOOTING

Problem	Possible Cause	Suggestion
Low yield of chromatin	Insufficient amount of samples.	To obtain the best results, the amount of samples should be 1 x10^6 to 5x10^6 cells, or 50 to 200 mg tissues per ChIP reaction.
	Insufficient chromatin extraction.	Ensure that all reagents have been added with the correct volume and in the correct order based on the sample amount.
		Check for sample lysis under microscope after the tissue/cell lysis step.
		Ensure that the cell or tissue species are compatible with this extraction procedure.
	Lysis or extraction reagents have expired. Expired reagents may cause inefficient extraction.	Ensure that the kit has not exceeded the expiration date of the kit. Standard shelf life, when stored properly, is 6 months from date of receipt.
	Incorrect temperature and/or insufficient incubation time during extraction.	Ensure the incubation time and temperature described in the protocol are followed correctly.
Degradation of chromatin	Improper storage of chromatin.	Chromatin sample should be stored at –80°C (3-6 months). Avoid multiple freeze/thaw cycles.

RELATED PRODUCTS

Chromatin Shearing and Cleanup
P-1006 DNA Concentrator Kit
P-2023 ChromaFlash™ Chromatin Isolation and Shearing Kit

Sonication Instruments
EQC-1100 EpiSonic™ Multi-Functional Bioprocessor 1100

ChIP Reaction
P-2025 ChromaFlash™ One-Step ChIP Kit
P-2026 ChromaFlash™ One-Step Magnetic ChIP kit

PCR Analysis
P-1029 ChIP-flash Quantitative PCR Fast Kit

ChIP-Grade Antibodies
A-1001 DNMT1 Monoclonal Antibody [60B122.1]
A-1003 DNMT3A Polyclonal Antibody
A-1004 DNMT3B Polyclonal Antibody

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A-1006	MBD1 Polyclonal Antibody
A-1007	MBD2 Polyclonal Antibody
A-1008	MBD3 Polyclonal Antibody
A-1009	MBD4 Polyclonal Antibody
A-1010	MGMT Polyclonal Antibody
A-1012	MeCP2 Polyclonal Antibody
A-1014	5-Methylcytosine Monoclonal Antibody [33D3]
A-2021	RING1 Polyclonal Antibody
A-2029	ENX1 Polyclonal Antibody
A-2030	ENX2 Polyclonal Antibody
A-2032	RNA Polymerase II Monoclonal Antibody [CTD4H8]
A-3009	SUV39H1 Monoclonal Antibody
A-3010	SUV39H2 Polyclonal Antibody
A-4001	HDAC1 Monoclonal Antibody
A-4002	HDAC2 Monoclonal Antibody
A-4003	HDAC3 Monoclonal Antibody
A-4004	HDAC4 Polyclonal Antibody
A-4005	HDAC5 Polyclonal Antibody
A-4006	HDAC6 Polyclonal Antibody
A-4007	HDAC7 Polyclonal Antibody
A-4008	HDAC8 Monoclonal Antibody
A-4009	HDAC9 Polyclonal Antibody
A-4012	PCAF Polyclonal Antibody
A-4013	GCN5 Polyclonal Antibody
A-4020	p300 Polyclonal Antibody
A-4021	Acetyl Histone H3 (K9/14) Polyclonal Antibody
A-4022	Acetyl Histone H3K9 Polyclonal Antibody
A-4023	Acetyl Histone H3K14 Polyclonal Antibody
A-4024	Acetyl Histone H3K18 Polyclonal Antibody
A-4025	Acetyl Histone H3K23 Polyclonal Antibody
A-4026	Acetyl Histone H3K56 Polyclonal Antibody
A-4027	Acetyl Histone H4K5 Polyclonal Antibody
A-4028	Acetyl Histone H4K8 Polyclonal Antibody
A-4031	Histone H3K4 Monomethyl Polyclonal Antibody
A-4032	Histone H3K4 Dimethyl Polyclonal Antibody
A-4033	Histone H3K4 Trimethyl Polyclonal Antibody
A-4034	Histone H3K9 Monomethyl Polyclonal Antibody
A-4035	Histone H3K9 Dimethyl Polyclonal Antibody
A-4036	Histone H3K9 Trimethyl Polyclonal Antibody
A-4037	Histone H3K27 Monomethyl Polyclonal Antibody
A-4038	Histone H3K27 Dimethyl Polyclonal Antibody
A-4039	Histone H3K27 Trimethyl Polyclonal Antibody
A-4040	Histone H3K36 Monomethyl Polyclonal Antibody
A-4041	Histone H3K36 Dimethyl Polyclonal Antibody
A-4042	Histone H3K36 Trimethyl Polyclonal Antibody
A-4043	Histone H3K79 Monomethyl Polyclonal Antibody
A-4044	Histone H3K79 Dimethyl Polyclonal Antibody
A-4045	Histone H3K79 Trimethyl Polyclonal Antibody
A-4046	Histone H4K20 Monomethyl Polyclonal Antibody
A-4047	Histone H4K20 Dimethyl Polyclonal Antibody
A-4048	Histone H4K20 Trimethyl Polyclonal Antibody
A-4049	Phospho-Histone H3 (Ser10) Monoclonal Antibody
A-4050	Phospho-Histone H3 (Ser28) Polyclonal Antibody

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